SIMILARITIES BETWEEN DAILY FLUCTUATIONS IN BACKGROUND RADIATION AND O_2–CONSUMPTION IN THE LIVING ORGANISM [1, 2]

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Recent studies on fluctuations in O_2-consumption and in spontaneous activity in conditions constant with respect to all factors known to influence organisms, have provided strong evidence that some external fluctuating physical factors are still exerting an influence on protoplasmic systems. The studies were made in conjunction with an analysis of temperature-independent, solar-day and lunar-day, cycles under constant conditions. Solar-day cycles have been known for a number of years to be widespread among organisms, and more recently it has become evident that lunar-day cycles also occur.

The evidence for an influence of an external factor has come from the recent rediscovery (see Stewart, 1898, for early literature) of correlations of organismic activities with barometric pressure and its changes (Brown, Freeland and Ralph, 1955; Brown, Webb, Bennett and Sandeen, 1955; Brown, Bennett, Webb and Ralph, 1956). These correlations have recently also been shown to occur in two lag-lead relationships. One is between the *rates* of barometric pressure change at certain specific times of day n, n-1, and n-2 as correlated with biological activity at an approximately corresponding time on day n. A second correlation is between the organismic activity at a particular time of day, expressed either in absolute terms or as deviation from the daily mean and the mean daily barometric pressure of the second day thereafter. That these correlations are in no manner responses to pressure changes themselves is clear not only from the lead-correlation of the organism on barometric pressure, but also from studies in which organisms were shielded from the normal external pressure fluctuations for as long as three consecutive months.

Recent work (Figge, 1947; Brown, Bennett and Ralph, 1955) has suggested that some form of cosmic radiation might be capable of influencing organisms. This view has been strengthened by the discovery of 27-day organismic fluctuations (Brown, Bennett, Webb and Ralph, 1956), a frequency recently reported to exist also in fluctuations in cosmic radiation (Simpson, 1954). As a consequence, the following studies were undertaken to investigate in some detail any possible relationships between general background radiation and organismic metabolism.

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Materials and Methods

The O_2-consumption of potatoes was recorded continuously from February 1 through May 31 at Evanston, Illinois by means of Brown (1954) recording respirometers modified in such a manner (Brown, 1957), that a constant pressure was maintained by hermetically sealing the respirometers and recording system in rigid copper containers, the barostats, in which the pressure was kept at a constant reduced level of 28.50 inches Hg. Five barostats, each with four respirometers jointly providing a single continuous record of the fluctuations in rate of O_2-consumption, were in essentially continuous operation during the four-month period.

A cylindrical core of potato with an eye was placed in each respirometer. These cores were about 2.2 cm. in diameter and 1½ cm. high. The first lot was prepared on January 31, and with the exception of a very few occasional single-potato replacements continued in the respirometers until May 1 when a completely new set of potatoes was substituted. These latter were followed through the month of May. Therefore, the first lot of potatoes remained in constant conditions including pressure for three months except for brief periods of 15–20 minutes once every two to six days when the O_2 reservoirs were being refilled and the CO_2-absorbent renewed. The second lot remained in constant conditions, with no replacements for one month.

Only complete, uninterrupted calendar-days of recordings were used in the analysis. Partial days of data (days a respirometer was set up) were discarded.

Background radiation was recorded continuously during the same four-month period by means of a 2×30-inch cosmic-ray counter with an appropriate scaler and data printer. This monitoring system, located in the same laboratory with the five barostat-respirometer ensembles, yielded a count rate of the order of 40,000/hour. A few days of data were missed about the middle of May.

Results

There were clear systematic fluctuations in O_2-consumption in the potatoes throughout the four-month period. These were most commonly ones appearing to possess a single major cycle a day. When 3×7-hour moving means of the average of all those two to five barostats for which recordings were complete on that day were calculated it was found that these daily fluctuations involved up to 28% increase, with a mean of 13.7% from lowest to highest values for the day for 30 sample days taken at random. In view of the leveling influence of the 3×7-hour sliding average, the actual range was undoubtedly substantially greater.

In Figure 1 (Nos. 1–5) are seen the mean forms of the daily fluctuation for the four-month period for each of the five barostats. It is quite evident that two general forms of mean daily fluctuation are apparent. Numbers 1, 3 and 4 showed a clear major cycle with a minimum in the early morning hours and a maximum in the late afternoon. Numbers 2 and 5 exhibited essentially a 180°-phase shift relative to the others. These five, independent, four-month samples, from lowest to highest values in the mean daily cycles are, respectively, 10%, 7.3%, 9.0%, 10%, and 4.8%. The average value of the five, 8.2%, is in remarkable agreement with the value, 8.0%, obtained for a two-month period in the spring of 1955 (Brown, 1957). All five mean cycles possess a minor peak about 6 P.M. and slight minima at 1–2 A.M. and 3 P.M., irrespective of the form of the major cycle. This fact is em-

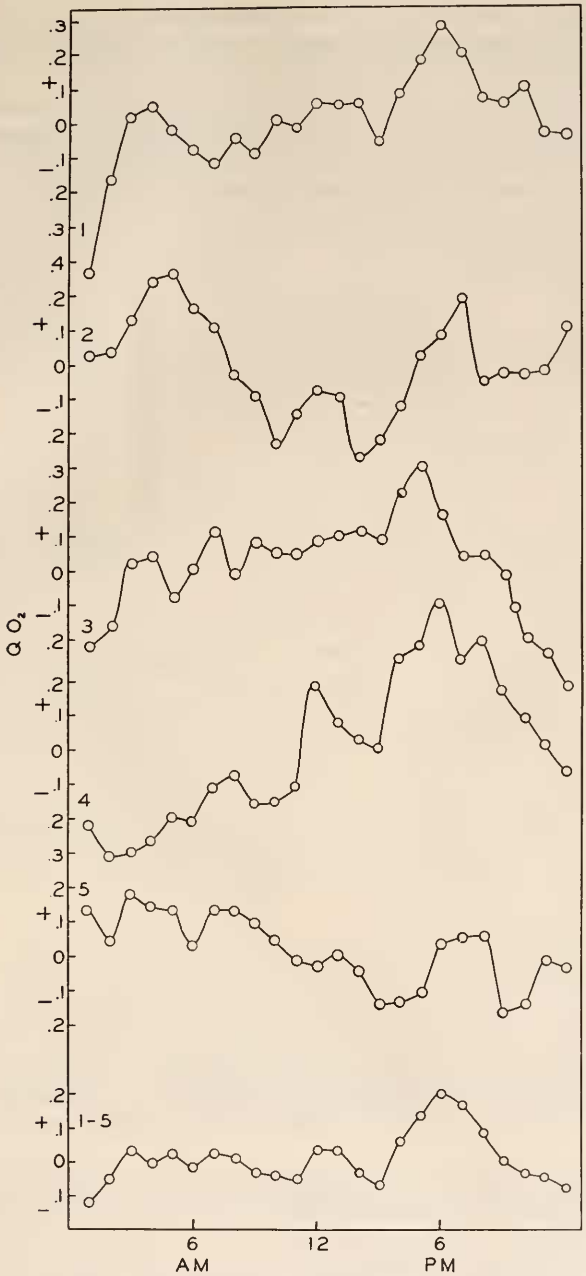

FIGURE 1. 1, 2, 3, 4, and 5 depict the mean daily cycles of fluctuation in O_2-consumption in potatoes, expressed as deviations from daily means, for each of the five independent respirometer-recording systems over the four-month period of study. 1–5 is the mean cycle for all the data. The actual percentage of the fluctuations is given in the text.

phasized in the mean 4-month cycle for all the barostats, Figure 1, (1–5) in which the 6 P.M. deviation from the daily mean is positive and highly significantly different from 0.

The mean daily cycle for all the barostats displayed no really significant evidence of a daily cycle except for the 5–6–7 P.M. peak clearly as a consequence of the algebraic summation of two forms of cycles, one essentially 180° out of phase with the other. It was apparent, also, from inspection of the single monthly mean

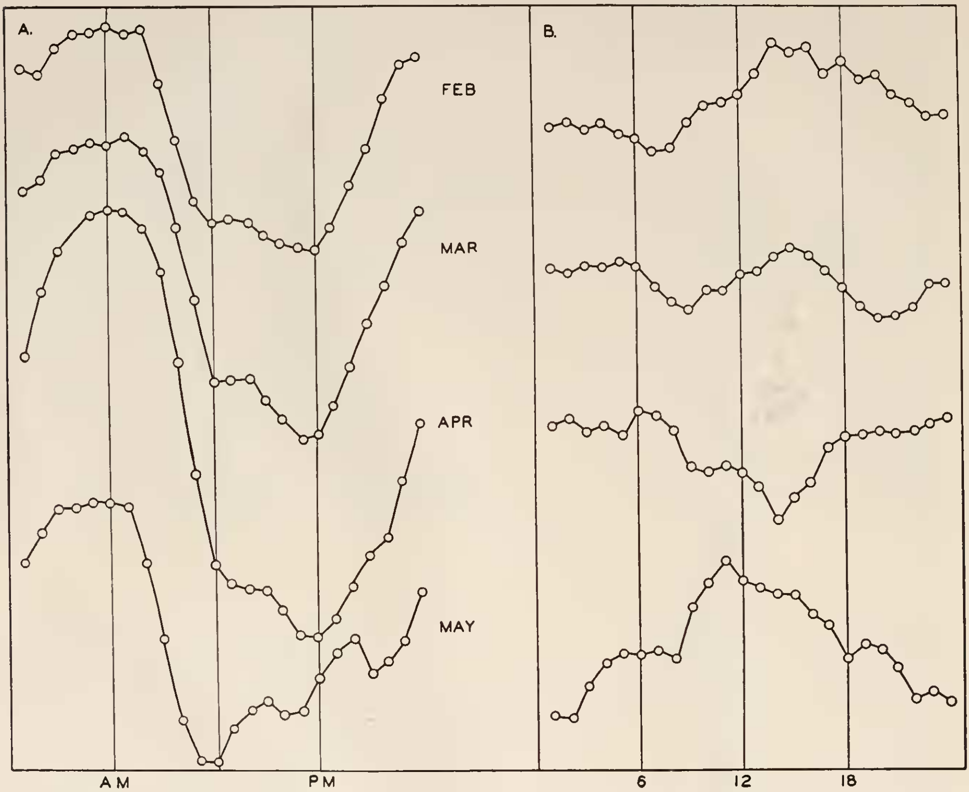

FIGURE 2. A. The mean daily cycles of general background radiation for each of four months. B. The mean lunar-day cycles of general background radiation for each of the four months. Lunar Zenith occurs at the 12th hour; Nadir, about 0 or 24. All these cycles are three-hour moving means of radiation. The mean percentages of the fluctuations are given in the text.

cycles for the single barostats that each of the five mean four-month cycles included monthly cycles tending to be of the form of the final four-month mean form, or 180° out of phase with it.

It seemed quite evident, therefore, that daily cycles of the potatoes were tending to exhibit one or the other of two forms, with, to the present, no suggestion that the occurrence of one type or the other is other than random.

An inspection of the fluctuations in radiation indicated there to be, in general, a clear mean daily cycle with a maximum about 6 A.M. and a minimum about 6

P.M. The mean daily cycles of three-hour moving means for each of the four months are seen in Figure 2 A. The mean cycles for February, March, and April are of strikingly similar form, with a gradually increasing amplitude (1.2%, 1.5%, and 2.3% increase from lowest to highest values). The maximum amplitude for a single day was about 10%. The cycle for May (5 days of data missing) showed an altered form though the amplitude, 1.3%, was of the same magnitude. Inspection of the daily data showed five days of the month (May 5, 15, 20, 23, 29) to have their cycles shifted about 180° relative to all the other days. The mean amplitude for the "typical" days was 2.2%, that for the five "shifted" days, 3.2%. Three days of the preceding month (April 1, 2, and 15) were also "shifted" days; no "shifted" days were present in February and March.

In view of the described presence of mean lunar-day cycles in numerous animals and plants, the mean lunar-day cycles of radiation were determined for each of the four months. These are seen in Figure 2 B. The cycles for February and March

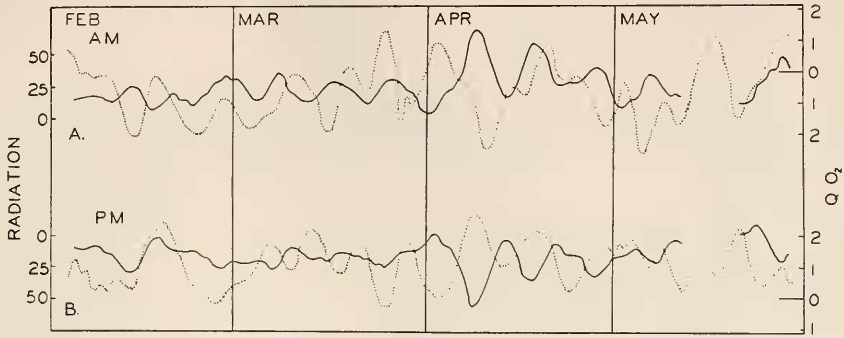

FIGURE 3. Five-day weighted (1:2:3:2:1) means of deviations of radiation intensity during the 2–6 A.M. and 2–6 P.M. periods from the daily means (solid lines) and corresponding weighted five-day means of O_2-consumption in potatoes for the 4–7 A.M. and 4–7 P.M. periods (dotted lines).

were rather similar, that of April shifted essentially 180° relative to the preceding two months. The cycle for May, with the highest amplitude of the four, again resembled those of February and March. There appeared to be a tendency for a maximum, or a minimum, to occur between the time of lunar Zenith and two to three hours afterwards, and for a maximum or a minimum to occur at the time of lunar Nadir. The amplitudes of the four average monthly cycles are, respectively, from minimum to maximum values, 0.57%, 0.37%, 0.60%, and 0.83%.

Correlation Between Radiation and O_2–Consumption

It is quite evident that even if the potato possesses mean daily cycles of O_2-consumption, its apparent tendency towards 180° phase-shifting would obscure much of this when large quantities of data were averaged. In view, however, of the relatively large-amplitude, daily fluctuations of the mean rates for all those potatoes recorded on a single day, an attempt was made to learn whether there might be a correlation between the amplitude of the day-by-day fluctuation in the background radiation and the amplitude of the day-by-day fluctuation in O_2-consumption of the potatoes.

In Figure 3 A is shown a weighted (1:2:3:2:1) five-day moving mean of the deviations in intensity of radiation from its daily mean for the 2–6 A.M. period (all were positive values), and similar deviations from the daily means for the 2–6 P.M. period (all were negative values). Plotted on different ordinate scales are

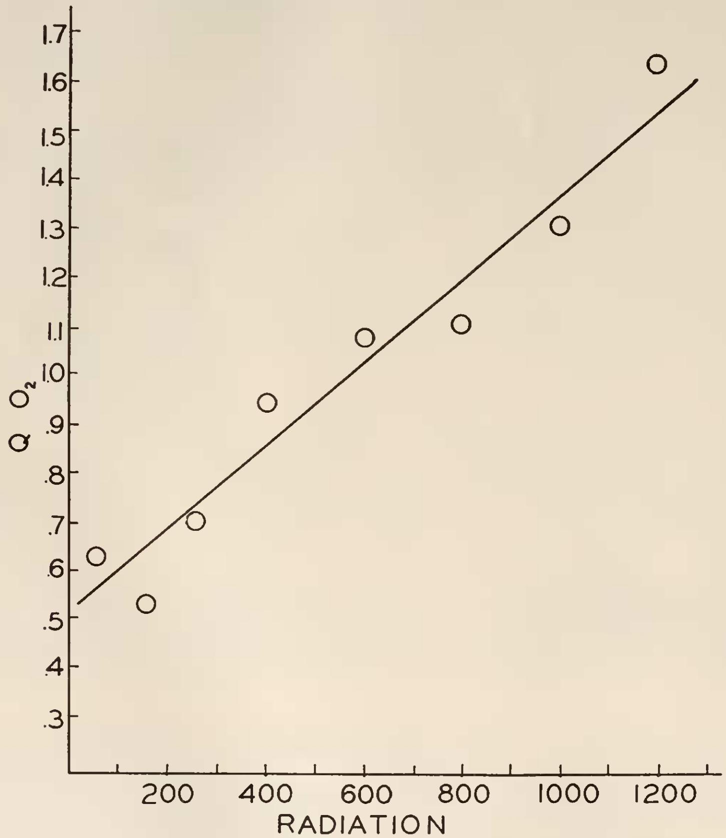

FIGURE 4. The relationship between the deviation in O_2-consumption at 4–7 A.M. and 4–7 P.M. from the daily mean of day *n*, expressed as deviations from monthly means, and the square of the deviation of radiation at 2–6 A.M. and 2–6 P.M. from its daily means for day *n*-1 expressed in the same terms ($P < 10^{-6}$).

superimposed correspondingly weighted five-day moving means of the deviation of O_2-consumption from the daily means for the 4–7 A.M. and 4–7 P.M. periods. It is evident from inspection that there is a highly suggestive similarity between the fluctuations in radiation and in O_2-consumption if one admits that the organismic cycles display alterations in sign of their correlation from time to time.

To quantify this similarity and to obtain at least an approximate measure of the significance of such an apparent similarity, a coefficient was determined for the correlation between the deviations of radiation from its mean monthly deviations for each of the two times of day, on the one hand, and the deviations of O_2-consumption from their mean monthly deviations, for these times of day on the other. In this correlation, the signs of the deviations were ignored. A study of the regressional relationship indicated the relationship to be non-linear, and that the deviation in O_2-consumption was, instead, linearly related to the square of the deviation in radiation, and that the deviation in O_2-consumption on day *n* showed its correlations with day *n*-2, and especially *n*-1, of radiation with a rapid drop in coefficient to days *n*-3 and *n*. The coefficients and their errors for days *n* through *n*-3 were, respectively, 0.191 ± 0.066, 0.337 ± 0.062, 0.290 ± 0.064, and 0.135 ± 0.068.

The calculated regression for O_2-consumption on day *n* on the square of radiation for day *n*-1 is seen in Figure 4. The relationships between deviations in radiation and in O_2-consumption were calculated to be as follows:

Radiation	QO_2
0.5%	0.3%
1.0%	1.8%
1.5%	4.1%
2.0%	7.0%

The lag-lead relationship, radiation day *n*-1, was apparently simply the best compromise between radiation on day *n*-2 when using only the 4–7 A.M. value of O_2-consumption (0.41 ± 0.081), and radiation on day *n* using only the 4–7 P.M. value (0.369 ± 0.084).

An inspection of the form of the fluctuations in radiation and in O_2-consumption clearly suggested that in no other lag-lead relationship would correlations significantly different from zero be found over the four-month period. However, this was investigated more specifically. Correlations were found between radiation and O_2-consumption as follows: between the 4–7 A.M. deviation in O_2-consumption on day *n* from its mean 13-day deviation for this time of day, and the deviation of the 4–7 P.M. (day *n*-2) to 4–7 A.M. (day *n*-1) change in radiation from its mean monthly change for this period the coefficient was 0.352 ± 0.087. On the other hand, between the corresponding 4–7 P.M. deviation in O_2-consumption for day *n* and the corresponding 4–7 A.M. to 4–7 P.M. change in radiation of day *n* the coefficient was 0.382 ± 0.083. Eleven other lag-lead relationships for each of the times of day, sampling from day *n*-30 to day *n* + 15 for radiation failed to yield any correlations similarly highly significantly different from zero.

Discussion

The results which have just been described provide one additional kind of evidence in support of the conclusion reached by Brown, Freeland and Ralph (1955) and Brown, Webb, Bennett and Sandeen (1955), in a study of correlations between metabolism of various organisms and concurrent rates and directions of barometric pressure change, that the organism in "constant conditions" still is responding to fluctuations in some external physical factor or factors. This view was given further support by the studies of Brown, Bennett, Webb and Ralph (1956) on the quahog. In more recent studies (Brown, 1957) employing barostats as in the current study, correlations with barometric pressure were found for O_2-consumption in potatoes over about a 1½-month period of study. In this latter investigation, unlike in earlier ones, there was a strong indication that the fluctuations in O_2-consumption on any given day tended to display either one of two patterns, one tending to be 180° out of phase with the other for either the whole or part of the day.

In the last-mentioned study there was a correlation of the deviations (ignoring sign) from the daily mean of the O_2-consumption of the potato at 4–7 A.M. on day *n* with the rate and direction of barometric pressure change from 2 to 6 A.M. centered on day *n*-2. In another kind of analysis of the data, there was found to be for the potato, a correlation between the 4–7 P.M. deviation from the daily mean of O_2-consumption and the mean barometric pressure on day $n + 2$ (Brown, Webb and Macey, 1957). Despite these correlations, the directly effective factor, in view of the use of barostats, could not have been pressure *per se*.

It is interesting that in this current four-month study there was also clearly reproduced a positive correlation between the deviation of the 4–7 P.M. value of O_2-consumption on day *n* from its daily mean and the mean barometric pressure of day $n + 2$ (Brown, Webb and Macey, 1957). It will be recalled that the 4–7 P.M. period was the only period of the day possessing a non-inverting cyclic component, and the correlation with barometric pressure correspondingly remained positive throughout the whole four-month period. In the spring of 1954, when a comparable and relatively striking negative correlation (-0.65) was found using the potato (not in barostats), it was similarly only the 4–7 P.M. period of the day which possessed this property.

Another notable observation made in the current studies is that the correlation with the deviations in radiation were maximum for days *n* to *n*-2(*n*-2, for the 4–7 A.M. O_2-consumption) for radiation. This is essentially the same lead-lag relationship found for the potato in the spring of 1955, a year earlier, with rates of barometric pressure change for comparable periods of the day, and also which obtained during the summer of 1955 for the sea weed, Fucus (unpublished results).

It seems reasonable to postulate that the living organism is displaying a mean one- to two-day lag response to some external factor correlated both with fluctuations in barometric pressure and with daily cycles in background radiation, and that the effective external fluctuations are in some manner correlated with the mean daily barometric pressure on the third to fourth day thereafter. From the standpoint of a possible significance of these external factors for the maintenance of the precision of the many known regular daily cycles observed to persist for long periods under constant conditions, it must be admitted that the organism can exhibit a metabolic response to some external factor which has clear mean daily cycles, even though with a randomly fluctuating amplitude. It has been postulated earlier (Brown, 1957) that organisms, through an endogenous capacity to oscillate, are able to maintain in many instances an endogenous cycle of the same frequency as the external ones.

The mean lunar-day cycles of radiation are of special interest relative to lunar-day mean cycles of biological activities which have been described (*e.g.*, Brown, Freeland and Ralph, 1955; Brown, Webb, Bennett and Sandeen, 1955; Brown, Shriner and Ralph, 1956). The principal maximum (or minimum) in both the mean lunar-day cycles of radiation and of most of those biological activities so far described appear to occur at the times of lunar Zenith or shortly afterwards. The results encourage a more detailed study of lunar-day relationships of radiation and activity comparable to the current one for solar-day relationships. Such a study is in progress. Also, very suggestive in this regard is the observation that the ratio of amplitude of the mean solar-day cycles to the lunar-day ones of radiation, is of the same order as the ratio of the amplitude of the solar-to-lunar-day cycles of most of the reported mean cycles for organisms, namely 2 or 3 to 1.

Summary

1. O_2-consumption of potatoes was recorded continuously through the four-month period, February-May, 1956.

2. There were daily fluctuations in rate which, even using 3×7-hour moving means, displayed a mean amplitude of about 14%.

3. Five independent respirometer-recording systems yielded mean daily cycles for the four-month period ranging in amplitude from 4.8 to 10.0% with a mean of 8.2%.

4. The mean cycles were of two forms, one essentially 180° out of phase with the other.

5. Solar-day and lunar-day mean fluctuations in background radiation were also determined for the period of the investigation.

6. A small but very highly significant correlation existed between the fluctuations in amplitude of the daily cycles in radiation on day n-1 and amplitude of the daily fluctuations in O_2-consumption in the potato on day n.

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